Distinguishing diffuse alopecia areata (AA) from pattern hair loss (PHL) using CD3$^+$ T cells

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Background: Distinguishing between diffuse subacute alopecia areata (AA), in which the peribulbar infiltrate is absent, and pattern hair loss is challenging, particularly in cases that lack marked follicular miniaturization and a marked catagen/telogen shift.

Objective: We sought to distinguish diffuse AA from pattern hair loss using CD3$^+$ T lymphocytes.

Methods: A total of 28 cases of subacute AA and 31 cases of pattern hair loss were selected and a 4-mm punch biopsy was performed. All the specimens were processed using the "HoVert" (horizontal and vertical) technique. In all cases, hematoxylin-eosin and immunohistochemical stains for CD3, CD4, CD8, and CD20 were performed.

Results: The presence of CD3$^+$ lymphocytes within empty follicular fibrous tracts (stela), even without a concomitant peribulbar infiltrate, is a reliable histopathological clue in supporting a diagnosis of AA (sensitivity 0.964, specificity 1, $P \leq .001$).

Limitations: Limited tissue for analysis remained in the clinical sample tissue blocks.

Conclusion: The presence of CD3$^+$ T-cells within empty follicular fibrous tracts (stela) supports a diagnosis of AA. (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2015.12.011.)

Key words: alopecia areata; CD3; follicular fibrous tracts; pattern hair loss; stela.

Distinguishing diffuse alopecia areata (AA) and female or male pattern hair loss (PHL) may be challenging because both are characterized histopathologically by follicular miniaturization and an increased catagen/telogen shift. Clinicians are often confronted with the differential diagnosis among diffuse AA, PHL, and chronic telogen effluvium (CTE), especially when diffuse hair loss occurs over androgen-dependent areas. The frequent coexistence of PHL and CTE further complicates this distinction, because both PHL and CTE are characterized by global shedding, which is often accentuated in the frontal and mid scalp areas.

Abbreviations used:

AA:	alopecia areata
AAI:	alopecia areata incognita
CTE:	chronic telogen effluvium
PHL:	pattern hair loss
T:V:	terminal:vellus

For a histologic distinction, there are clues to specific diagnoses. CTE does not have follicular miniaturization. AA, PHL, and CTE, however, all have empty follicular fibrous tracts (so-called "empty stela" or "streamers") because of either follicular

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miniaturization or a catagen/telogen shift.[1] The miniaturized follicles are not long enough to extend down the fibrous tracts into the reticular dermis and subcutis. Similarly, the follicular epithelium of a catagen/telogen follicle resides only in the papillary dermis, with the subjacent tract being empty. In a minority of cases of AA, some lymphocytes may persist in the empty follicular fibrous tracts (stela).[2-4] Further complicating diagnostics, melanin may also be present in the fibrous tracts in AA, and even pigment casts may be identified, especially in people with dark hair.[1,5] This creates the potential for a misdiagnosis of trichotillosis (trichotillomania or traction alopecia). Fortunately, follicular miniaturization does not occur in CTE and trichotillosis.[6]

CAPSULE SUMMARY

- Without peribulbar lymphocytes, distinguishing between alopecia areata and pattern hair loss is challenging because both possess follicular miniaturization and a catagen/telogen shift.
- We describe a new tool to help overcome this diagnostic challenge.
- The presence of $CD3^+$ lymphocytes within empty follicular fibrous tracts (stela) reliably favors alopecia areata over pattern hair loss.

The presence of a peribulbar lymphocytic infiltrate, the so-called "hive of bees," sometimes admixed with eosinophils, confirms a diagnosis of AA. However, in subacute AA, where the peribulbar infiltrate is usually absent, an appropriate diagnosis of AA must be based on marked follicular miniaturization and a marked catagen/telogen shift.[3] A marked catagen/telogen shift, particularly more than 50%, and profound follicular miniaturization, with a terminal:vellus (T:V) ratio of greater than 1:7, are diagnostic of subacute AA.[2,7] However, when the T:V ratio is somewhere between 1:1 and 1:7, and the catagen/telogen shift is between 20% and 50%, a histologic distinction between AA and PHL is not possible.

The presence of a T-cell lymphocytic infiltrate in AA is well established.[4] However, the distribution and number of these T cells is not well documented in subacute AA, because the T-cell infiltrate is often sparse or absent on a simple hematoxylin-eosin examination. In this study, we characterized the inflammatory cell infiltrate in both AA and PHL in an attempt to identify reliable histopathological clues that can distinguish these entities. Here we report the use of the CD3 antigen, which is found on all mature T lymphocytes.

METHODS

Selection of cases

A total of 28 cases of subacute AA and 31 cases of PHL were selected.

The selected cases diagnosed as AA had to meet the following criteria: (1) a characteristic clinical presentation; (2) a confirmed histopathological diagnosis confirmed by 2 hair-loss dermatopathologist experts; and (3) histopathological findings with at least 1 of the following conditions: (a) the presence of a peribulbar lymphocytic infiltrate; (b) a catagen/telogen shift of more than 50%; or (c) marked follicular miniaturization with a T:V ratio of <1:7. Other findings, such as the presence of so-called "nanogen" hairs,[3] lymphocytes and melanin in empty follicular fibrous tracts (stela), eosinophils in the tracts and around follicular bulbs, and dilated follicular openings were also taken into consideration. Cases with both a T:V ratio between 1:1 and 1:7 and catagen/telogen shift between 20% and 50% were included if there was a peribulbar lymphocytic infiltrate. In cases AA2 and AA4 only vertical sections were obtained so the T:V ratio and catagen/telogen percentage could not be established, but these were included because of the presence of a peribulbar infiltrate.

The selected cases diagnosed as PHL had to meet the following criteria: (1) a characteristic clinical presentation; (2) a confirmed histopathological diagnosis by 2 hair-loss dermatopathologist experts; and (3) histopathological findings including all 3 of the following conditions: (a) a decreased T:V hair ratio between 1:1 to 1:3 for patients younger than 50 years of age, and 1.5:1 to 1:3 for patients older than 50 years (no distinction between PHL and senescence was made for patients age >50 years); and (b) an increased percentage of catagen/telogen-phase hairs between 10% to 30%; and (c) an absence of a peribulbar lymphocytic infiltrate on hematoxylin-eosin examination. An additional histopathological finding taken into consideration was increased variability of follicle diameter within the same examined section.

In all cases, a diagnosis of CTE alone was ruled out by the presence of follicular miniaturization. Trichotillomania and traction alopecia were ruled out because of clinical findings and the absence of a trichomalacia, pigment casts, and a distorted follicular architecture. In cases with a marked catagen/telogen shift, a diagnosis of syphilis was also excluded by the absence of a plasma cell-rich

Table I. Patients diagnosed with alopecia areata or pattern hair loss

Patient no.	Sex	Age, y	Clinical differential diagnosis	Telogen/ vellus hair	Telogen + catagen hair	CD3 in follicular fibrous tracts	CD3 peribulbar	CD3 peri-infundibular	CD3 subcutaneous	CD3 reticular dermis	CD3 papular dermis	CD3 epidermis
AA1	F	31	AA	1	30%	1+	2+	3+	Trace	1+	2+	1+
AA2	F	30	AA	NA	NA	2+	0	0	Trace	1+	3+	1-2+
AA3	F	37	AA-LPP-LE	1	20%	1+	1+	2-3+	1+	2-3+	2+	2-3+
AA4	F	22	AA	NA	NA	2+	2+	2+	1+	1+	2+	Trace
AA5	F	26	AA	1/2	70%	1+	1+	2+	Trace	2+	2+	0
AA6	F	31	AA	<1/7	50%	1+	1+	2+	1+	2-3+	2-3+	1+
AA7	F	35	AA	1/5	30%	3+	2+	2-3+	2+	2-3+	2-3+	0
AA8	F	54	AA-CTE	1/9	40%	2+	1+	1-2+	Trace	2-3+	1+	0
AA9	M	58	AA	<1/7	30%	1+	3+	1+	1+	3+	1+	0
AA10	F	32	AA-LE	1/4	20%	2+	0	2+	Trace	1+	2-3+	Trace
AA11	F	18	AA	1/5	30%	1+	0	1+	Trace	Trace	1+	0
AA12	F	56	LPP	<1/7	30%	1+	1	2-3+	1+	2+	2-3+	Trace
AA13	F	7	AA	1/10	35%	2+	3+	2-3+	1+	2-3+	2-3+	1+
AA14	F	45	AA	<1/7	25%	2-3+	1+	3+	2+	2+	3+	1+
AA15	F	80	AA-CTE	1/4	40%	No stella identified	3+	3+	Trace	3+	3+	1+
AA16	F	50	AA	<1/7	30%	2-3+	2+	3+	0	2+	2-3+	1+
AA17	F	63	AA	<1/7	100%	3+	3+	1+	2+	2-3+	1+	Trace
AA18	F	58	AA	<1/7	40%	2+	2-3+	3+	Trace	2+	2+	Trace
AA19	F	35	AA	<1/7	30%	2+	2-3+	2+	Trace	2-3+	2+	Trace
AA20	F	57	AA	<1/7	50%	2+	1-2+	1-2+	1+	2+	1-2+	0
AA21	F	59	AA	1/9	30%	2+	2-3+	1-2+	No subcutis present	2-3+	1-2+	1+
AA22	F	38	AA-LE	1/3	50%	2-3+	2-3+	2+	2+	2-3+	2+	1+
AA23	F	54	AA-LE	1/5	20%	2-3+	1+	2+	Trace	2+	2+	2+
AA24	F	57	AA	<1/7	30%	3+	2-3+	2+	1+	2-3+	1-2+	Trace
AA25	F	27	AA	<1/7	40%	2+	3+	2-3+	No subcutis present	3+	2+	2+
AA26	F	44	AA-CTE	<1/7	30%	2+	0	2+	Trace	2+	2+	Trace
AA27	F	59	AA-LE	<1/7	35%	2+	2+	Trace-1+	No subcutis present	2+	1+	Trace
AA28	F	38	AA	1/4	20%	2+	2+	Trace	Trace	1-2+	1+	0
PHL1	F	55	PHL-LE	1/2	20%	0	0	Trace	0	No reticular dermis present	1+	Trace

Continued

Table I. Cont'd

Patient no.	Sex	Age, y	Clinical differential diagnosis	Telogen/ vellus hair	Telogen + catagen hair	CD3 in follicular fibrous tracts	CD3 peribulbar	CD3 peri-infundibular	CD3 subcutaneous	CD3 reticular dermis	CD3 papular dermis	CD3 epidermis
PHL2	M	30	PHL-FFA	1/3	10%	0	Trace-1%	2+	No subcutis present	1+	2-3+	2+
PHL3	F	43	PHL-AA	1/1	15%	0	0	2+	1+	1+	2+	Trace
PHL4	F	19	PHL-AA	1	10%	Trace-1+	0	1-2+	Trace	1+	2-3+	1-2+
PHL5	M	25	PHL-FFA	NA	NA	0	0	2+	0	Trace	2+	1+
PHL6	F	28	PHL-AA	1	15%	Trace	0	2+	Trace	1+	2+	Trace
PHL7	M	16	PHL-AA	1/2	10%	0	0	2+	Trace	No reticular dermis present	2-3+	1+
PHL8	F	65	PHL-LPP	1	5%	Trace	0	2+	Trace	Trace	2+	0
PHL9	F	39	PHL-LPP	1.5/1	0%	0	0	0	Trace	1-2+	Trace	0
PHL10	F	46	PHL-CTE-AA	1/2	20%	Trace	0	Trace	0	2+	2+	0
PHL11	F	72	CTE-SEN-LPP	1/1	20%	0	0	1-2+	0	0	1-2+	Trace-1+
PHL12	M	20	PHL-LPP	1/2.5	20%	Trace	0	Trace-1+	0	1+	1+	Trace
PHL13	F	63	CTE-LPP-PHL	1/2	14%	0	0	2+	0	0	2+	1+
PHL14	F	28	CTE-PHL	1/3	20%	0	0	2+	0	1+	2+	0
PHL15	F	36	CTE-PHL	1/1.5	20%	0	0	Trace-1+	0	Trace	Trace-1+	0
PHL16	F	59	LPP	1/1	25%	0	0	1-2+	0	0	1-2+	0
PHL17	F	30	CTE-PHL-AA	1/1.5	30%	0	0	1+	0	0	1+	1+
PHL18	F	63	LPP	1/1.5	10%	0	0	2+	0	0	2+	Trace
PHL19	F	56	CTE-LPP-PHL	1/2	15%	0	0	2+	0	Trace	2+	1+
PHL20	F	35	PHL-LPP	1/1	10%	Trace	0	2+	Trace	1+	2+	0
PHL21	F	39	CTE-SEBDERM	1/2	5%	0	0	2+	0	Trace	2+	0
PHL22	F	47	CTE-LPP-PHL	1/1	15%	0	0	2+	0	0	2+	Trace
PHL23	F	29	AA-PHL	1/1	5%	0	0	1-2+	Trace	Trace	1-2+	1+
PHL24	F	63	CTE-LPP	1/1	20%	0	0	1+	0	Trace	1+	0
PHL25	F	79	LPP	1/1	15%	0	0	3+	0	Trace	3+ (Actinic keratosis)	3+ (Actinic keratosis)
PHL26	F	68	PHL-CTE-LPP	1/1	20%	0	0	2+	0	0	2+	0
PHL27	F	43	PHL-SEBDERM-LPP	1/1	10%	0	0	2+	0	0	2+	Trace
PHL28	F	55	CTE-LPP-PHL	1.5/1	10%	0	0	1+	0	0	1+	0
PHL29	F	39	PHL-CTE	1/1	15%	0	0	Trace-1+	0	0	Trace-1+	Trace
PHL30	F	79	PHL-SEN-LPP	1/1	25%	0	0	Trace	0	0	Trace	0
PHL31	F	49	PHL-CTE-LPP	1/2	10%	Trace	0	2+	0	Trace	2+	0

All missing values were counted as negative for AA.

AA, Alopecia areata; *CTE*, chronic telogen effluvium; *F*, female; *FFA*, frontal fibrosing alopecia; *LE*, lupus erythematosus; *LPP*, lichen planopilaris; *M*, male; *NA*, not applicable; *PHL*, pattern hair loss; *SEBDERM*, seborrheic dermatitis; *SEN*, senescence.

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infiltrate and a negative serologic analysis. Lupus erythematosus was excluded in patients with a marked catagen/telogen shift because of the clinical presentation, negative serologic studies, and an absence of interfollicular or follicular interface change.

Processing of specimens

In all cases a 4-mm punch biopsy was performed. All the specimens were processed using the recently described "HoVert" (horizontal and vertical) technique.[8] To do this, each biopsy specimen was transected horizontally (transversely) approximately 1 mm below the skin surface to create an epidermal disc and a lower portion. The epidermal disc was then bisected and embedded vertically, thereby allowing visualization of the entire interfollicular epidermis, the dermoepidermal junction, and the papillary dermis. The dermal/subcutaneous portion of the biopsy specimen was bisected horizontally (transversely) at the dermosubcutaneous junction. Both cut surfaces were inked, and the inked surfaces were embedded down. Immunohistochemical staining was performed on unstained, 5-μm sections placed on charged slides. The slides were processed in an automated BenchMark ULTRA staining module (Ventana Medical Systems Inc, Tucson, AZ). An appropriate titer for the CD3, CD4, CD8, and CD20 antibodies was predetermined using a College of American Pathologists-approved protocol, and a positive control was included on the same slide. The automated staining involved 74 steps beginning with deparaffinization at 72°C and ending with a final slide rinse and coverslipping.

Examination of specimens

In all cases, hematoxylin-eosin and immunohistochemical stains for CD3, CD4, CD8, and CD20 were evaluated by 2 hair-loss expert dermatopathologists. On initial review, only the CD3 showed significant, quantifiable staining, so CD4, CD8, and CD20 were not quantified. The density of the $CD3^+$ T-cell infiltrate was assessed using grading of 0, trace, 1+, 2+, and 3+. T-cell density was analyzed both as a score (0, trace = 0.5, 1 = 1, 1-2 = 1.5, 2 = 2, 2-3 = 2.5, 3 = 3) and as a 5-point ordinal (0, trace, 1, 2, 3). This density was assessed in the epidermis, papillary dermis, reticular dermis, subcutis, peri-infundibular dermis, peribulbar dermis, and empty follicular fibrous tracts (stela) (Table I).

All statistical analysis was performed by the Biostatistics and Design Program of the Oregon Clinical and Translational Research Institute with Stata 13.1 (StataCorp LP, College Station, TX). Confidence intervals were calculated using the Jeffreys method described by Brown et al.[9]

RESULTS

Both the AA and PHL groups consisted of more female than male patients (male:female = 1:27 [AA] and 4:27 [PHL]). The average age of the patients was similar in AA and PHL groups (42.9 vs 45.7 years).

In the AA cases, a clinical diagnosis of AA was in the differential diagnosis in 27 of 28 cases. Other diagnoses in the clinical differential were CTE in 3 cases, lupus in 5 cases, and lichen planopilaris in 2 cases. In the PHL cases, PHL was in the differential diagnosis in 25 of 31 cases. Other diagnoses in the clinical differential were CTE in 14 cases, AA in 7 cases, senescence of aging in 2 cases, seborrheic dermatitis in 2 cases, lupus in 1 case, and lichen planopilaris in 19 cases (in 2 of which the frontal fibrosing variant of lichen planopilaris was considered).

Both CD3 analysis methods as a score and as a 5-point ordinal gave the same conclusions. Unreadable results were grouped with 0 or trace counts for calculating sensitivity and specificity. Table II summarizes the estimates of sensitivity, being defined as the probability of greater than or equal to 1+ CD3 count (vs 0, trace, or unreadable) when the diagnosis is AA, and specificity, being defined as the probability of 0, trace, or unreadable count (vs ≥1+) when the diagnosis is PHL. The sensitivity and specificity results are summarized best in Fig 1.

The most reliable histopathological finding is the presence of $CD3^+$ lymphocytes within the empty follicular fibrous tracts (stela) with concomitant peribulbar $CD3^+$ lymphocytes (sensitivity 1.000, specificity 1.000, $P \leq .001$), or isolated $CD3^+$ lymphocytes within empty follicular fibrous tracts (stela) even without a concomitant peribulbar infiltrate (sensitivity 0.964, specificity 1.000, $P \leq .001$) (Figs 2 and 3). The presence of $CD3^+$ **[F2-4/C]** lymphocytes within empty follicular fibrous tracts **[F3-4/C]** (stela) is a significantly more sensitive finding when compared to the peribulbar CD3 counts (sensitivity 0.964 vs 0.857, $P \leq .001$), but they both share the same specificity (specificity 1.000, $P \leq .001$). The presence of $CD3^+$ lymphocytes within the reticular dermis is also a finding with high sensitivity (0.964, $P \leq .001$), but low specificity (0.710, $P \leq .001$). Subcutaneous $CD3^+$ lymphocytes have a high specificity (0.968, $P \leq .001$), but a low sensitivity (0.429, $P \leq .001$). Epidermal (sensitivity 0.429, specificity 0.710, $P = .291$), papillary dermal (sensitivity 0.964, specificity 0.129, $P = .356$),

Table II. Sensitivity and specificity results based on the location of the $CD3^+$ lymphocytes

Location of $CD3^+$ lymphocytes	Sensitivity*	95% CI†	Specificity‡	95% CI†	P§
In fibrous tracts	0.964	0.845-0.996	1.000	0.923-1.000	<.001
Peribulbar	0.857	0.695-0.950	1.000	0.923-1.000	<.001
Reticular dermis	0.964	0.845-0.996	0.710	0.537-0.846	<.001
Subcutaneous	0.429	0.260-0.611	0.968	0.859-0.996	<.001
Epidermis	0.429	0.260-0.611	0.710	0.537-0.846	.291
Peri-infundibular	0.893	0.741-0.969	0.226	0.107-0.393	.306
Papillary dermis	0.964	0.845-0.996	0.129	0.045-0.278	.356
Combinations					
In fibrous tracts or peribulbar	1.000	0.915-1.000	1.000	0.923-1.000	<.001
In fibrous tracts or subcutaneous	0.964	0.845-0.996	0.968	0.859-0.996	<.001
In fibrous tracts or epidermis	1.000	0.915-1.000	0.710	0.537-0.846	<.001
In fibrous tracts or reticular dermis	1.000	0.915-1.000	0.710	0.537-0.846	<.001
In fibrous tracts or peri-infundibular	1.000	0.915-1.000	0.226	0.107-0.393	.011
In fibrous tracts or papillary dermis	1.000	0.915-1.000	0.129	0.045-0.278	.114

Practically, sensitivity depicts the probability of a positive test when the diagnosis is alopecia areata (positive predictive value), whereas specificity depicts the probability of a negative test when the diagnosis is not alopecia areata (negative predictive value).
CI, Confidence interval.
*Probability of ≥1+ CD3 count (vs 0, trace, or unreadable) in alopecia areata.
†By Jeffreys method.[9]
‡Probability of 0, trace, or unreadable CD3 count (vs ≥1+) in pattern hair loss.
§From Fisher exact test of association between alopecia areata and having ≥1+ CD3 counts.

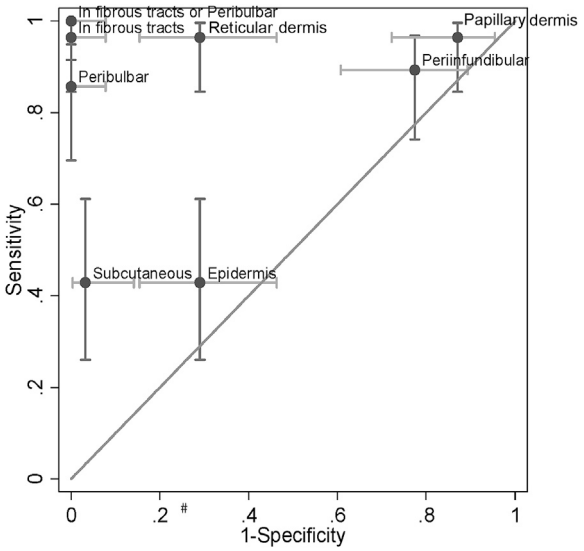

Fig 1. Summary of the sensitivity and specificity results based on the location of the $CD3^+$ lymphocytes. The vertical axis indicates increased sensitivity and the horizontal axis indicates decreased specificity (1 − specificity). The points in the upper left corner therefore reflect the perfect classification, whereas the points below or near the diagonal line indicate the least discriminative findings. The sensitivity and specificity confidence intervals are also indicated, respectively, with a vertical and horizontal line around the central point.

and peri-infundibular (sensitivity 0.893, specificity 0.226, P = .306) CD3 distribution lacks both sensitivity and specificity.

DISCUSSION

We demonstrate that the presence of an increased number of $CD3^+$ T cells in the mid to reticular dermis and the subcutis, especially in empty follicular fibrous tracts (stela), provides strong evidence for a diagnosis of AA in cases where PHL is also a clinical and histologic consideration. The presence of $CD3^+$ lymphocytes in the tracts has a high sensitivity and specificity even without a peribulbar infiltrate. This novel application of immunohistochemistry is of significant use because traditional hematoxylin-eosin sections alone often do not allow a distinction between these 2 entities, and the density and location of the lymphocytic infiltrate is often not obvious on hematoxylin-eosin sections alone. Increased $CD3^+$ lymphocytes are even identified in this pattern in cases of AA without any apparent lymphocytic infiltrate on hematoxylin-eosin examination.

On a low-power magnification, the reticular dermal/subcutaneous distribution of $CD3^+$ lymphocytes seen in AA is a helpful clue but with limited sensitivity. The presence of papillary dermal $CD3^+$ lymphocytes, however, does not have any diagnostic value. This may be explained by the frequent association of mild seborrheic dermatitis, which may be accompanied by a superficial lymphocytic infiltrate. Mild peri-infundibular chronic inflammation is especially common in African American women and may be regarded as normal.[1]

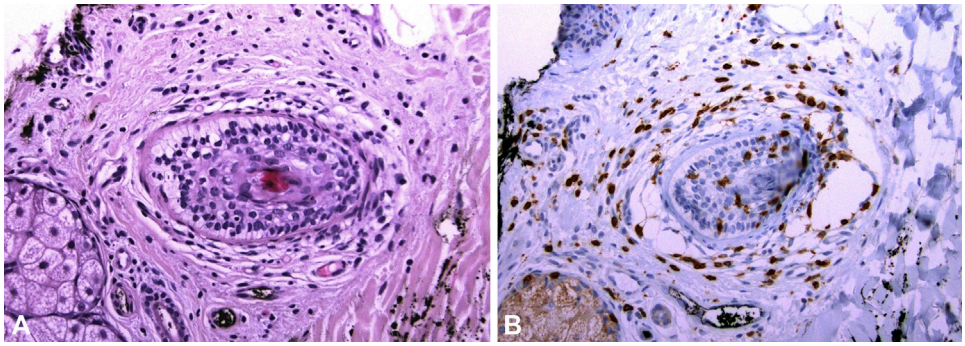

Fig 2. Alopecia areata. **A**, Hematoxylin-eosin stain: sparse peribulbar lymphocytic infiltrate. **B**, CD3 immunostain of the same follicle. Lymphocytes are identified inside the follicle, which are not apparent on hematoxylin-eosin stain.

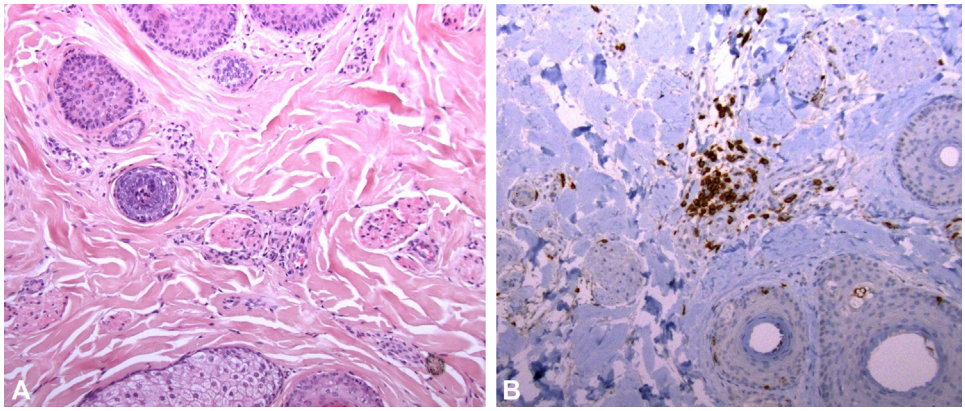

Fig 3. Alopecia areata. **A**, Hematoxylin-eosin stain: empty follicular tract (stela). **B**, CD3 immunostain of empty follicular tract (stela) with lymphocytes that are not apparent on hematoxylin-eosin stain.

Epidermal changes, such as case PHL25 in which we also observed an actinic keratosis, could explain the presence of intense superficial infiltrate.

Although PHL and AA represent very different pathophysiologic processes, they both cause varying degrees of follicular miniaturization. In both processes, as the miniaturization progresses, the length of the follicular cycle shortens, and the ratio of catagen and telogen follicles increases. The presence of a significant catagen/telogen shift results is an even greater diagnostic dilemma, because CTE enters the diagnostic differential. Of note, follicular miniaturization in PHL, especially in men, marches forward until the follicles disappear completely, leaving empty follicular fibrous tracts (stela) with no follicular epithelium. The decrease in total follicular counts with stabilizing of the T:V ratio in severe PHL suggests that, in contrast to AA, miniaturization does not stop with a vellus follicle but progresses to follicular deletion. In these cases, a biopsy specimen may show a low follicular density.[10] AA has a similar process because the ongoing autoimmune attack dramatically shortens the follicular cycle. Indeed, marked miniaturization may occur in a very short period in AA. With unchecked, active inflammation, the percentage of follicles in the catagen/telogen phase may approach 100%.[1,2,5] It is the more moderate cases, though, that are difficult to distinguish from PHL, for which we demonstrate the use of CD3.

The presence of T lymphocytes within the mononuclear infiltrate present in AA is already well documented. $CD8^+$ cells are more likely to be present within the follicular epithelium than $CD4^+$ lymphocytes.[1] Animal model studies conclude that although CD8 cells are the direct modulators of hair loss, the CD4 cells play a classic helper role in AA onset. Specific HLA class II alleles are important through antigen presentation to CD4 cells for the onset and progression of AA.[4] As a part of this study, we also examined CD4, CD8, and CD20 in all of the specimens. However, a comparison of B- and T-cell populations has limited diagnostic value because the infiltrate is too sparse in subacute AA and PHL. In addition, it is not possible to quantify the CD4:CD8 ratio. Thus, CD3

density assessment proves to be the only easily reproducible diagnostic tool in distinguishing PHL from AA.

Characterization of the recently reported entities "alopecia areata incognita" (AAI) and "acute hair miniaturization" may be especially helped by the use of CD3 immunostaining. It is not clear yet whether AAI and acute hair miniaturization are the same entity, because both show acute, diffuse hair thinning with only yellow dots and short regrowing hairs on dermoscopy.[11] The presence of circumscribed patches of hair loss are one useful clinical clue for AAI.[12] Some do not agree that acute hair miniaturization belongs within the spectrum of AA because of the frontal accentuation of the hair loss, a feature more associated with PHL. Of note, biopsy specimens of both AAI and acute hair miniaturization are particularly challenging because of the complete absence of a peribulbar lymphocytic infiltrate.[5,13,14] Some of the reported histopathologic clues for AAI include the presence of dilated infundibular openings and small aggregates of basaloid cells, corresponding to small telogen follicles, but these are nonspecific findings and may also be seen in AA, PHL, and CTE.[13] Perhaps our application of CD3 immunostaining will help better define both AAI and acute hair miniaturization.

Another application of CD3 immunostaining may be in the distinction between permanent alopecia after chemotherapy from PHL and AA. Although biopsy specimens of permanent alopecia after chemotherapy are quite similar to PHL and AA, permanent alopecia after chemotherapy is a distinctive alopecia because it is irreversible but not cicatricial (scarring) in nature.[15] As in PHL and AA, histopathology findings in permanent alopecia after chemotherapy are similar to PHL and AA with follicular miniaturization and a catagen/telogen shift.[15-18] The presence of basaloid structures resembling telogen-phase follicles has been described as a unique feature,[16,17] but these may actually be altered telogen-phase follicles after chemotherapeutic insult.[17] Although PHL may be distinguished clinically from permanent alopecia after chemotherapy because of the presence of diffuse, short, sparse hair in PHL, AA may be impossible to differentiate from permanent alopecia after chemotherapy, and CD3 immunostaining may be quite helpful in this clinical situation.

Conclusion

CD3 immunostaining is a useful tool in distinguishing AA from PHL in the absence of an obvious peribulbar hive of bees infiltrate on hematoxylin-eosin sections. The presence of T cells within empty follicular fibrous tracts (stela) and a reticular dermal/subcutaneous, so-called "bottom-heavy" distribution strongly supports a diagnosis of AA, whereas an absence of T cells within the tracts strongly favors PHL.

REFERENCES

1. Sperling LC. *An atlas of hair pathology with clinical correlations*. 2nd ed. Boca Raton (FL): Taylor and Francis Group; 2012.
2. Whiting DA. Histopathologic features of alopecia areata: a new look. *Arch Dermatol*. 2003;139:1555-1559.
3. Peckham SJ, Sloan SB, Elston DM. Histologic features of alopecia areata other than peribulbar lymphocytic infiltrates. *J Am Acad Dermatol*. 2011;65:615-620.
4. Alkhalifah A, Alsantali A, Wang E, McElwee K, Shapiro J. Alopecia areata update. *J Am Acad Dermatol*. 2010;62:177-188.
5. Dy LC, Whiting DA. Histopathology of alopecia areata, acute and chronic: why is it important to the clinician? *Dermatologic Therapy*. 2013;24:369-374.
6. Stefanato CM. Histopathology of alopecia: a clinico-pathological approach to diagnosis. *Histopathology*. 2010;56:24-38.
7. Aslani FS, Dastgheib L, Banihashemi BM. Hair counts in scalp biopsy of males and females with androgenetic alopecia compared with normal subjects. *J Cutan Pathol*. 2009;36:734-739.
8. Nguyen JV, Hudacek K, Whitten JA, Rubin AI, Seykora JT. The HoVert technique: a novel method for the sectioning of alopecia biopsies. *J Cutan Pathol*. 2011;38:401-406.
9. Brown LD, Cai TT, DasGupta A. Interval estimation for a binomial proportion. *Statistical Science*. 2001;16:101-133.
10. Messenger AG, Sinclair R. Follicular miniaturization in female pattern hair loss: clinicopathological correlations. *Br J Dermatol*. 2006;155:926-930.
11. Tosti A, Whiting D, Iorizzo M, et al. The role of scalp dermoscopy in the diagnosis of alopecia areata incognita. *J Am Acad Dermatol*. 2008;59:64-67.
12. Park J, Song K-H, Nam K-H. Circumscribed alopecia areata incognita. *Australas J Dermatol*. 2013;54:52-54.
13. Miteva M, Misciali C, Fanti PA, Tosti A. Histopathologic features of alopecia areata incognito: a review of 46 cases. *J Cutan Pathol*. 2012;39:596-602.
14. Quercetani R, Rebora AE, Fedi MC, et al. Patients with profuse hair shedding may reveal anagen hair dystrophy: a diagnostic clue of alopecia areata incognita. *J Eur Acad Dermatol Venereol*. 2011;25:808-810.
15. Miteva M, Misciali C, Fanti PA, Vincenzi C, Romanelli P, Tosti A. Permanent alopecia after systemic chemotherapy: a clinicopathological study of 10 cases. *Am J Dermatopathol*. 2011;33:345-350.
16. Tallon B, Blanchard E, Goldberg L. Permanent chemotherapy-induced alopecia: case report and review of the literature. *J Am Acad Dermatol*. 2010;63:333-336.
17. Tallon B, Blanchard E, Goldberg L. Permanent chemotherapy-induced alopecia: histopathologic criteria still to be defined. *J Am Acad Dermatol*. 2013;68:151-152.
18. Choi M, Kim MS, Park SY, et al. Clinical characteristics of chemotherapy-induced alopecia in childhood. *J Am Acad Dermatol*. 2014;70:499-505.